

BOX PCT

ATTORNEY'S DOCKET NO: 24259

U.S. DEPARTMENT OF COMMERCE, PATENT AND TRADEMARK OFFICE		DATE: <u>07</u> June 2000 (<u>07</u> .06.2000)
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLN. NO. (if known): <u>09/555964</u> No Yea Assign
INTERNATIONAL APPLICATION NO.: PCT/IL98/00592	INTERNATIONAL FILING DATE: 7 December 1998 (07.12.98)	PRIORITY DATE CLAIMED: 7 December 1997 (07.12.97)
TITLE OF INVENTION: SKIN TEST FOR SCHIZOPHRENIA		
APPLICANT(S) FOR DO/EO/US: SHINITZKY, Meir; DECKMANN, Michael		
Applicant hereby submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<p>1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the time limit set in 35 USC 371(b) and PCT Articles 22 and 39(1).</p> <p>4. <input type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)): <ul style="list-style-type: none"> a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> has been transmitted by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US) </p> <p>6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</p> <p>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ul style="list-style-type: none"> a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. </p> <p>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</p> <p>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</p> <p>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p>		
ITEMS 11. TO 16. BELOW CONCERN OTHER DOCUMENT(S) OR INFORMATION INCLUDED:		
<p>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input checked="" type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment</p> <p>14. <input type="checkbox"/> A substitute specification.</p> <p>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>16. <input checked="" type="checkbox"/> TRANSMITTAL FORM; FEE CALCULATION; INTERNATIONAL PUBLICATION WO 99/30163 INTERNATIONAL PUBLICATION DATE 17 JUNE 1999; APPLICATION CONSISTING OF 18 PAGES INCLUDING; 1 COVER PAGE CONTAINING THE ABSTRACT; 13 PAGES TEXTUAL SPECIFICATION, 3 PAGES OF 10 CLAIMS; 1 SHEET OF DRAWINGS; PCT/IPEA/416 NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT; PCT/IPEA/409 INTERNATIONAL PRELIMINARY EXAMINATION REPORT WITH AMENDED SHEETS (SPECIFICATION AND CLAIMS) TO BE EXAMINED; PRELIMINARY AMENDMENT TO IPER TO BE EXAMINED; UNEXECUTED INVENTOR'S DECLARATION; PCT/ISA/220 NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT; PCT/ISA/210 INTERNATIONAL SEARCH REPORT; PCT/IPEA/408 WRITTEN OPINION DATED 30 SEPTEMBER 1999; RESPONSE TO WRITTEN OPINION DATED 14 FEBRUARY 2000.</p>		

U.S. APPLICATION NO. (if known) 09/555964	INTERNATIONAL APPLICATION NO. PCT/IL98/00592	DATE: 07 June 2000 (07.06.2000)	
17. <input checked="" type="checkbox"/> The following fees are submitted:		<u>CALCULATIONS</u>	<u>PTO USE ONLY</u>
Basic National Fee (37 CFR 1.492(a)(1)-(5): Search Report has been prepared by the EPO or JPO:.....\$840.00		\$ 840.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482).....\$670.00			
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....\$760.00			
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....\$970.00			
International preliminary examination fee (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....\$ 96.00			
ENTER APPROPRIATE BASIC FEE AMOUNT = \$ 840.00			
Surcharge of \$130.00 for furnishing the oath or declaration later than <u>20</u> <u>30</u> months from the earliest claimed priority date (37 CFR 1.492(e)).		\$	
CLAIMS	NO. FILED	NO. EXTRA	RATE
TOTAL	8=	0	X \$ 18.00
INDEPENDENT	4 - 3=	10	X \$ 78.00
Multiple dependent claims(s) (if applicable)		+ \$260.00	\$ 0.00
		TOTAL OF ABOVE CALCULATIONS =	\$ 918.00
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).		\$	0.00
		SUBTOTAL =	\$ 918.00
Processing fee of \$130.00 for furnishing the English translation later than <u>20</u> <u>30</u> months from the earliest claimed priority date (37 CFR 1.492(f)). +		\$	0.00
		TOTAL NATIONAL FEE =	\$ 918.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +		\$	0.00
		TOTAL FEES ENCLOSED =	\$ 918.00
		Amount to be: refunded _____ charged	\$ _____

U.S. APPLICATION NO. (if known)	INTERNATIONAL APPLICATION NO.	DATE: 07 June 2000 (07.06.2000)
09/555964	PCT/IL98/00592	

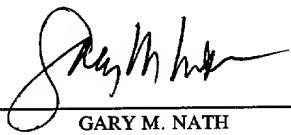
- a. ONE check in the amount of \$918.00 to cover the above fees is enclosed.
- b. Please charge my Deposit Account No. 14-0112 in the amount of \$_____ to cover the above fees. (A duplicate copy of this sheet is enclosed.)
- c. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0112.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed to request that the application be restored to pending status.

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Rev. 02/98

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533 Rec'd PCT/PTO 07 JUN 2000

BOX PCT
Attorney Docket No. 24259

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

SHINITZKY, Meir; DECKMANN, Michael

International Application No. PCT/IL98/00592

Serial No. NOT YET ASSIGNED

International Filing Date: 7 December 1998 (07.12.98)

Filed: June 7, 2000

For: **SKIN TEST FOR SCHIZOPHRENIA**

PRELIMINARY AMENDMENT

The Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Before calculating the filing fee for the above identified application, please enter the following amendments:

IN THE CLAIMS:

Please cancel claims 1-5 with out prejudice. Please amend the following claim.

Claim 10, line 1, delete "any one of the previous claims" and insert in lieu thereof --claim 6--

Please add the following claims:

--11. The method of claim 7, wherein said proteins or fractions thereof have a pI within the range of above 6.5 to about 9.5.--

--12. The method of claim 8, wherein said proteins or fractions thereof have a pI within the range of above 6.5 to about 9.5.--

--13. The method of claim 9, wherein said proteins or fractions thereof have a pI within the range of above 6.5 to about 9.5.--

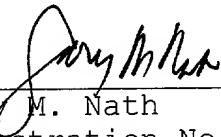
REMARKS

The above amendments have been made to remove multiple dependencies from the claims, and no new matter has been added. Claims 11-13 have been added to compensate for the subject matter deleted from heretofore multiple dependent claim 10. No new matter has been added.

Respectfully submitted,

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By:


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1 / PACT

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SKIN TEST FOR SCHIZOPHRENIA

FIELD OF THE INVENTION

The present invention relates to diagnostic tests, and more specifically to diagnostic tests for psychiatric diseases.

5 LIST OF REFERENCES

The following is a list of references which is considered to be pertinent for the purpose of understanding the background of the invention:

1. Nyland, H., Naess, A. and Lundre, H.: *Lymphocyte subpopulations in peripheral blood from schizophrenic patients.* Acta Psychiat. Scand., 1980; 61; 313-318.
2. Hirata-Hibi, M., Higashi, S., Tachibana, T. and Watanabe, N., *Stimulated lymphocytes in schizophrenia* Arch. Gen. Psychiat. 1982; 39; 82-87.
3. Coeffey, C.E., Sullivan, J.L. and Rice, R.R., *T lymphocytes in schizophrenia*. Biol. Psychiatry 1983; 18; 113-119.
4. Kolyaskina, G.I. *Blood lymphocytes in schizophrenia - immunological and virological aspects.* Adv. Biol. Psychiat. 1983; 12; 142-149.
5. Bessler, H., Eviatar J., Meshulan. M., Tyano. S., Djaldetti, M. and Sirota P. *Theophyllin-sensitive T-lymphocyte subpopulation in schizophrenic patients.* Biol. Psychiatr. 1987; 22; 1025-1029.
6. Muller, N., Ackenheil, M., Eckstein, R., Hofschuster, E. and Mempel,

W. Reduced suppressor cell function in psychiatric patients. Ann. N.Y. Acad. Sci. 1987; 396; 686-690.

7. Mihalovic, L.J. and Jankovic B.D. *Effects of intraventricularly injected anti-caudatus antibody on the electrical activity of the cat brain. Nature 1961; 192; 665.*

8. Rapport, M.M., Karplak, S.E. and Mahadik, S.P. *Biological activities of antibodies injected into the brain. Fed. Proc. 1979; 38; 2391.*

9. Vartanian, M.E. Doyskina, G.S. Lozovsky, D.V., Burbaera, G.S. and Ignaton, S.A. *Aspects of humoral and cellular immunity in schizophrenia. In: Birth Defects. Original Article Series D. Bergsma and A. Goldstein, eds. vol 14; 339-364; Alan R. Liss; New York, N.Y; 1978.*

10. Rotman, A. *Blood platelets in psychopharmacological research. Prog. Neuropsychopharmacol. 1983; 6; 135-151.*

11. Pletscher, A. *Biological Psychiatry*, Gea Racagni, ed; Elsevier Science Publisher; 1991; 2, 354-356.

12. Shinitzky, M., Deckmann, M., Kessler, A., Sirota, P., Rabbs, A. and Elizur, A.. *Platelet autoantibodies in dementia and schizophrenia - possible implications for mental disorders. Ann. N.Y. Acad. Sci. 1991; 621; 205-217.*

13. Kessler, A. and Shinitzky, M., *Platelets from schizophrenic patients bear autoantibodies that inhibit dopamine uptake. Psychobiology 1993; 21; 229-306.*

14. PCT Patent Application WO 95/23970.

15. Shinitzky, M. *et al.*, WO 97/13152.

16. Deckmann *et al.*, *Italian Journal of Psychiatry and Behavioural Sciences, 6:29-34, 1996.*

The above references will be acknowledged in the text below by indicating their number from the above list shown in brackets.

BACKGROUND OF THE INVENTION

Schizophrenia is a syndrome which encompasses a variety of symptoms including paranoia, auditory hallucination, delusions, catatonia, bizarre behavior and emotional withdrawal. Schizophrenia affects about 1% of the total population. Its economical and social burden on society is enormous since onset occurs in youth thus requiring patients to be under medical and psychiatric supervision for most of their lives. Schizophrenia is therefore one of the most costly diseases in the industrialized world.

Since the biochemical basis of schizophrenia has not yet been elucidated, diagnosis today is still based solely upon psychiatric evaluation. Furthermore, no therapy is currently available for schizophrenia although the symptoms may be ameliorated by neuroleptic drugs.

Many reports have implicated the immune system in the etiology and course of several mental disorders. Serum antibodies which cross-react with brain antigens have been found in the blood of schizophrenic patients⁽¹⁻⁶⁾, thus indicating that schizophrenia is also an autoimmune disease⁽⁷⁻⁹⁾. Furthermore, platelets have been used as a model for neuronal tissue^(10,11) and elevated levels of autoantibodies to platelets have been detected in schizophrenic and demented patients, but not in patients suffering from manic-depressive disorder, depression, personality disorders or schizoaffective disorders⁽¹²⁻¹⁴⁾. An assay for the diagnosis of multi-infarct dementia and dementia of the Alzheimer type was described based on detection of a high level of a platelet associated antibody⁽¹⁵⁾.

A cellular response against autologous platelets was also demonstrated in schizophrenia patients who showed a delayed type hypersensitivity (DTH) reaction when injected with platelets collected from their own blood⁽¹⁶⁾.

It is therefore the object of the present invention to provide a test for the diagnosis of schizophrenia in a subject.

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SUMMARY OF THE INVENTION

A novel finding in accordance with the invention is that schizophrenic patients exhibit a reaction which displays characteristics of a typical delayed-type hypersensitivity reaction when injected with a specific, 5 novel fraction of platelet proteins. This novel fraction is referred to herein as "Pool 2 proteins". The Pool 2 proteins, which constitute an aspect of the invention, are characterized by having an isoelectric point (pI) which is above about 6.5 and preferably within the range of about 6.5 to about 9.5. When injected with Pool 2 proteins, schizophrenic patients show such a 10 delayed-type hypersensitivity (DTH) reaction.

It is to be noted that although the reaction of the patients to the Pool 2 proteins is referred to above and below as a DTH reaction, there may be cases in which the time profile of the reaction of the patient to the Pool 2 proteins will be different than the time profile of a typical DTH reaction.

15 The present invention thus provides a protein preparation (hereinafter "*Pool 2 proteins*") comprising platelet derived proteins or fractions thereof having an isoelectric point (pI) above about 6.5 and preferably within the range of above 6.5 to about 9.5, said preparation capable of eliciting a DTH reaction in a schizophrenic individual upon injection 20 thereof to the individual.

The present invention further provides a diagnostic method for assaying schizophrenia in a subject comprising:

- (a) obtaining a preparation comprising, as an active component, platelet derived proteins or fractions thereof having an isolelectric point (pI) 25 above about 6.5 and preferably within the range of about 6.5 to about 9.5 ("*pool 2 proteins*");
 - (b) injecting said protein preparation into a subject; and
 - (c) examining the subject for the occurrence of a delayed type hypersensitivity reaction at the site of the injection, a positive result being a

reaction above that which is observed in non-schizophrenic subjects, indicating that the subject has a high likelihood of being schizophrenic.

In accordance with the invention it was also surprisingly found that the Pool 2 proteins may be either prepared from autologous platelets of 5 the individual to be tested or, alternatively, from a pool of heterologous platelets which were obtained from a number of individuals other than the tested subject. It was also surprisingly found that, in most cases, the DTH reaction in a schizophrenic patient is substantially higher when the individual is injected with such Pool 2 proteins obtained from a pool of heterologous 10 platelets as compared to a lower DTH reaction in the same tested individual injected with Pool 2 proteins prepared from his own autologous platelets. This finding provides the advantage of preparing a Pool 2 protein preparation which may then either be used immediately or alternatively, be stored at appropriate conditions (e.g. refrigeration) and used for various periods of time 15 to diagnose schizophrenia in a large number of individuals. This obviates the need to repeatedly obtain a blood sample comprising platelets from the tested individual immediately prior to carrying out the diagnostic assay of the invention.

Thus, by a preferred embodiment, the present invention further 20 provides a method for the preparation of a reagent for use in diagnosis of schizophrenia, comprising:

- (a) obtaining blood samples from a number of individuals, preparing a pool from said samples and collecting platelets therefrom;
- (b) preparing a protein fraction from said platelet preparation 25 comprising proteins or fractions thereof having a pI of above about 6.5, preferably within the range of about 6.5 to about 9.5.

The Pool 2 proteins prepared from heterologous individuals may either be prepared from a number of individuals suffering from schizophrenia or, alternatively, also from a mixture of blood samples obtained from schizophrenic patients as well as healthy individuals.

5 The present invention yet further provides a diagnostic method for assaying schizophrenia in a subject comprising:

(a) obtaining a blood sample from a number of schizophrenic and/or non schizophrenic individuals other than the tested subject and collecting platelets therefrom;

10 (b) preparing a protein fraction from said platelet separation comprising proteins or fractions thereof having a pI of above about 6.5, preferably within the range of above 6.5 to about 9.5;

(c) injecting said protein preparation into a subject; and

15 (d) examining the subject for the occurrence of a delayed type hypersensitivity reaction at the site of the injection, a positive result being a reaction above that which is observed in non-schizophrenic subjects, indicating that the subject has a high likelihood of being schizophrenic.

By a preferred embodiment, a method for diagnosis of schizophrenia in an individual is provided as above wherein the protein fraction collected in stage (b) is then stored at appropriate conditions for repetitive use as described in stages (c) and (d) above at later periods of time for a number of tested individuals.

20 Although, as explained above, there is an advantage in preparing a pool of Pool 2 proteins from a number of individuals other than the individual to be tested, in accordance with the diagnostic method of the invention, it is also possible to use a Pool 2 preparation prepared from autologous platelets of the individual to be tested.

The present invention thus provides a diagnostic method for assaying schizophrenia in a subject comprising;

- (a) obtaining a blood sample from an individual and collecting platelets therefrom;
- 5 (b) collecting proteins or fractions thereof from said platelet sample, said proteins or fractions having a pI of above about 6.5, preferably within the range of about 6.5 to about 9.5;
- (c) injecting said collected proteins or fractions thereof to the tested individual; and
- 10 (d) examining the subject for the occurrence of delayed type hypersensitivity reaction at the site of the injection, a positive result being a reaction above that which is observed in non-schizophrenic subjects, indicating that the subject has a high likelihood of being schizophrenic.

Typically, the Pool 2 proteins will be collected by subjecting the collected platelet proteins to isoelectric focusing wherein the proteins having a pI of above about 6.5, preferably within the range of 6.5 to 9.5 are collected by methods known in the art.

However, in accordance with the invention it has also been found that the Pool 2 protein preparation may also be prepared by methods which do not include isoelectric focusing such as, for example, by extracting the proteins from the platelet sample using for example a detergent, such extraction resulting in Pool 2 proteins having the desired pI values and capable of eliciting a DTH reaction in a schizophrenic patient.

If desired, the Pool 2 protein preparation obtained by any one of the methods mentioned above may be subject to further fractionation steps, e.g. by thin layer chromatography, by high pressure liquid chromatography or by many other purification fractionation methods known, *per se*. Fractions thus obtained can each then be tested for activity, namely for its ability to cause the DTH reaction in schizophrenic patients. Such purified fractions, as

well as individual proteins, polypeptides or peptides among the Pool 2 proteins which are active in eliciting the DTH reaction in schizophrenic patients, are also an aspect of the invention.

The Pool 2 protein preparation prepared in accordance with the invention and used in the diagnostic methods of the invention include proteins purified from the Pool 2 proteins, polypeptides or peptides comprising sequences of such proteins, fractions thereof, as well as proteins, polypeptides or peptides obtained by synthesis or by genetic engineering having a sequence identical to that of the proteins of the Pool 2 proteins.

In accordance with the invention, Pool 2 proteins used in the diagnostic assay of the invention are such which are capable of eliciting DTH activity in an injected individual, the DTH activity being tested by the test known in the art. In short, the Pool 2 proteins are intradermally injected into the tested individual at the forearm or thigh and the reaction at the injection site is evaluated after 24, 48 and 72 hours by measuring the reaction diameter around the induration. As mentioned above, there may be cases in which the time profile of the reaction will differ from the typical time profile of a DTH reaction.

The present invention further provides a kit for use in diagnosis of schizophrenia, comprising said Pool 2 proteins, active protein fractions obtained therefrom, or individual active proteins or peptides, derived from said Pool 2 proteins. Preferably, such proteins are provided in either injectable form or in a form suitable for preparing an injectable formulation, e.g. a lyophilysate. Typically, the kit will be provided with instructions for use or a chart or pictures for guidance of the manner of scoring the results.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows the reaction on the forearm of a schizophrenic patient which was injected with the following preparations:

- i. Pool 2 proteins (marked as P2)
- 5 ii. Pool 1 proteins (marked as P1)
- iii. Autologous platelets (marked A)
- iv. PBS (marked as C = control).

The invention will now be illustrated in the following examples, which are annexed to the above drawings.

10

EXAMPLES

A study on the reaction of a subject to an injection of platelets collected from his or her own blood or an injection of Pool 2 proteins was carried out in Israel at The Hospital for Mental Health in Sha'ar Menashe, The 15 Geriatric Hospital in Pardes Hana and at the Weizmann Institute of Science.

Example 1 Injection of autologous proteins

1. Preparation of platelets

10 ml venous blood was collected from a subject and 20 centrifuged for 20 mins. at 20°C and 150 g. The supernatant containing platelets was collected and centrifuged three times for 10 min. at 20°C and 2000 g and the platelets resuspended in phosphate buffered saline (PBS) containing 5 mM EDTA. After the last washing, the platelets were resuspended in sterile PBS at a final concentration of 2×10^8 platelets/ml.

25

2. Autologous skin test and measurement of delayed type hypersensitivity (DTH) reaction

0.1 ml of the platelet suspension obtained as above from a 30 particular subject was injected intradermally back into the forearm of the

same subject. A second injection of 0.1 ml PBS spaced about 10 cm from the point of sample injection served as a control.

DTH reaction at the injection sites was measured in accordance with methods known in the art (Skornik, Y., et al., *Cancer Immunol. Immunother.* **11**:93-96, 1981).

Results

The tests were carried out for groups subject:

10 a) 18 healthy subjects under the age of 65.
 b) 10 healthy subjects above the age of 65.
 c) 41 schizophrenic patients.
 d) 21 demented patients.

The results of this test are shown in the following Table 1.

Table 1

**Autologous (Self-Self) Skin Reaction Against Platelets in Humans
for the Detection of Schizophrenia**

Summary of a Multi-Center Study

Schizophrenic Persons n = 41	Non-schizophrenic Persons n = 49
Skin Reaction positive: 25	Skin Reaction positive: 0
Skin Reaction borderline: 13	Skin Reaction borderline: 1
Skin Reaction negative: 3	Skin Reaction negative: 48
Total	Total
Sensitivity: 92%	Specificity: 98%

As can be seen, 38 out of the 41 schizophrenic patients showed a DTH reaction, while only one healthy individual under the age of 65 out of the 18 which were tested, reacted positively. Positive reactions were not observed in 10 healthy individuals over the age of 65, as well as in 21 demented patients. Sensitivity of this test is thus 92% and the specificity is 98%.

As seen in the Table, while almost all of the tested schizophrenic patients showed a DTH reaction to the Pool 2 proteins prepared from their autologous platelets, only one healthy individual had a positive DTH reaction. Thus, the diagnostic assay of the invention showed a very high sensitivity and specificity for the diagnosis of schizophrenia in a tested individual.

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Example 2 Preparation of Pool 1 and Pool 2 proteins**Methods**

Platelet suspension containing about 20 gr total protein was obtained as in Example 1, and the platelets solubilized with 40 ml of a solution containing 0.5% of the detergents NP-40 and Triton-X-100 for 5 mins. at room temperature with gentle shaking. The solution was then centrifuged at 4000 g for 15 mins. at 20°C. The supernatant was collected, and the pellet was subjected to two further extractions with 10 ml 0.1% Triton-X-100. The three supernatants were combined and Bio-Lyte Ampholyte™ 3/10 (40%) of BioRad was added to a final concentration of 1%. The solubilized proteins were subjected to isoelectric focusing. 60 ml of sample was applied to the Rotofor™ system of BioRad, using 0.1 M phosphoric acid as anode solution, and 0.1 M NaOH as cathode solution. The isoelectric focusing was performed for about 4 hours at 10°C using 10 Watt constant power until the current remained constant for 30 mins. Proteins were divided into two separate groups in accordance with their pI: proteins having a pI in the range of 2-6.5 are referred to as Pool 1 proteins while proteins having a pI in the range of 6.5-9.5 are referred to as Pool 2 proteins. Pool 1 and Pool 2 proteins were harvested separately and diluted 1:50 with PBS.

Example 3 Injection of Pool 1 and Pool 2 proteins**Method**

The following four preparations were injected intradermally into the forearm of a schizophrenic patient:

- i. Pool 2 proteins (marked as P2)
- ii. Pool 1 proteins (marked as P1)
- iii. Autologous platelets (marked A)
- iv. PBS.

0.1 ml of each above preparation were injected and the preparations were injected at four different injection sites spaced about 10 cm from

Amendment

each other. The skin reaction at each injection site was monitored 24 h, 48 h and 72 h after injection.

Results

The results are seen in Fig. 1 where (i) is the highest injection site on the arm and (iv) is the lowest.

As seen in the figure, a DTH response measured as explained above, was observed in a schizophrenic patient at the site of injection of Pool 2 proteins and no DTH reaction was seen at the site of P1 injection. Furthermore, the DTH reaction at the P2 site of injection was substantially enhanced as compared to the DTH reaction seen at the site of injection of the autologous platelets. Thus, it is, in most cases, preferred to use a P2 protein preparation obtained from a pool of blood samples obtained from several heterologous individuals in the diagnostic assay of the invention.

Article 31

CLAIMS:

1. Use of a protein preparation comprising platelet derived proteins or fractions thereof having an isoelectric point (pI) above about 6.5 and preferably within the range of above 6.5 to about 9.5, for the preparation of an injectable reagent for diagnosis of schizophrenia in an individual by determining a Delayed Type Hypersensitivity (DTH) reaction in said individual following injection of said reagent to the individual.
2. A kit for use in diagnosis of schizophrenia in an individual by detection of DTH reaction in said individual, comprising:
 - (i) a protein or a fraction thereof prepared from human platelets, said proteins or fractions thereof having a pI of above about 6.5;
 - (ii) a chart and/or pictures for guidance of the manner of scoring said DTH reaction; and
 - (iii) instructions for use.
3. A kit in accordance with Claim 6, wherein the proteins or fractions thereof have a pI within the range of above 6.5 to about 9.5.
4. A kit in accordance with Claims 6 or 7, wherein the proteins or fractions thereof are prepared from heterologous platelets obtained from a number of individuals other than the individual to be tested.
5. A kit in accordance with Claims 6 or 7, wherein the proteins or fractions thereof are prepared from autologous platelets obtained from the individual to be tested.
6. A method for the preparation of a reagent for use in diagnosis of schizophrenia in an individual by detecting a DTH reaction in said individual following injection of said reagent to the individual, comprising:
 - (a) obtaining blood samples from a number of individuals, preparing a pool from said samples and collecting platelets therefrom;

(b) preparing a protein fraction from said platelet preparation comprising proteins or fractions thereof having a pI of above about 6.5.

7. A diagnostic method for determining schizophrenia in a subject comprising:

- (a) obtaining a preparation comprising, as an active component, platelet derived proteins or fractions thereof having a pI above about 6.5;
- (b) injecting said preparation into a subject; and
- (c) examining the subject for the occurrence of delayed type hypersensitivity reaction at the site of the injection, a positive result being a reaction above that which is observed in non-schizophrenic subjects, indicating that the subject has a high likelihood of being schizophrenic.

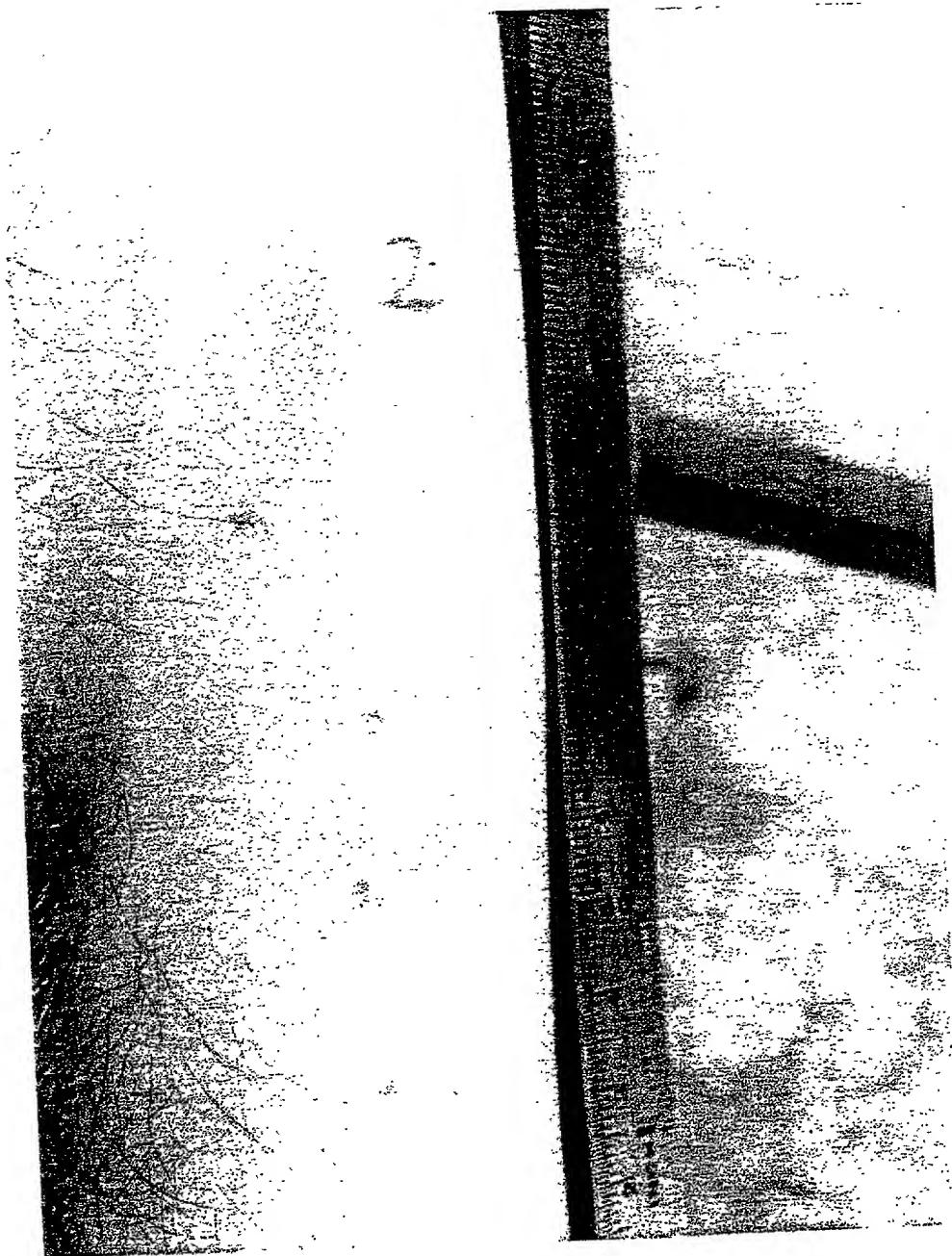
8. A diagnostic method for determining schizophrenia in a subject comprising:

- (a) obtaining a blood sample from a number of schizophrenic and/or non schizophrenic individuals other than the tested subject and collecting platelets therefrom;
- (b) preparing a protein fraction from said platelet separation comprising proteins or fractions thereof having a pI of above about 6.5;
- (c) injecting said protein preparation into a subject; and
- (d) examining the subject for the occurrence of a delayed type hypersensitivity reaction at the site of the injection, a positive result being a reaction above that which is observed in non-schizophrenic subjects, indicating that the subject has a high likelihood of being schizophrenic.

9. A diagnostic method for determining schizophrenia in a subject comprising;

- (a) obtaining a blood sample from an individual and collecting platelets therefrom;
- (b) collecting proteins or fractions thereof from said platelet sample, said proteins or fractions having a pI of above about 6.5.
- (c) injecting said collected proteins or fractions thereof to the tested individual; and
- (d) examining the subject for the occurrence of delayed type hypersensitivity reaction at the site of the injection, a positive result being a reaction above that which is observed in non-schizophrenic subjects, indicating that the subject has a high likelihood of being schizophrenic.

10. The method of any one of the previous claims, wherein said proteins or fractions thereof have a pI within the range of above 6.5 to about 9.5.



P₂

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Fig. 1

DECLARATION FOR PATENT APPLICATION

Attorney Docket: 24074
Page 1 of 1

As a below-named inventor(s), I/we hereby declare, that:

My/Our residence(s), post office address(es) and citizenship(s) is/are as stated below next to my/our name(s).

I/we believe I/we am/are the original inventor, first and sole (if only one name is listed below) or the original, first and joint inventors (if plural names are listed below) of the subject matter which is claimed, and for which a patent is sought on the invention entitled:

SKIN TEST FOR SCHIZOPHRENIA

the specification of which: (check one)

 is attached hereto. was filed on 7 December 1998, as Serial No. PCT/IL98/00592and was amended on 20 (if applicable).

We hereby state that we have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

We acknowledge the duty to disclose information which is material to the patentability of this application as defined by 37 CFR § 1.56.

We hereby claim foreign priority benefits under 35 U.S.C. § 119 of any foreign applications; for patent or inventor's certificate listed below, and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Applications:

<u>Serial No.</u>	<u>Country</u>	<u>Priority Claimed</u>
<u>(Application No.)</u>	<u>(Country)</u>	<u>(Day/Month/Year Filed)</u> <input checked="" type="checkbox"/> Yes <input type="checkbox"/>
<u>(Application No.)</u>	<u>(Country)</u>	<u>(Day/Month/Year Filed)</u> <input type="checkbox"/> Yes <input type="checkbox"/>
<u>(Application No.)</u>	<u>(Country)</u>	<u>(Day/Month/Year Filed)</u> <input type="checkbox"/> Yes <input type="checkbox"/>

We hereby appoint Gary M. Nath, Reg. No. 26,965; Harold L. Novick, Reg. No. 26,011; Todd G. Jugeau, Reg. No. 40,669; Lee C. Heiman, Reg. No. 41,827; Jerald L. Meyer, Reg. No. 41,194; Joshua S. Goldberg, Reg. No. 44,126; David Milligan, Reg. No. 47,893; David R. Murphy, Reg. No. 22,761; Paul A. Sanchez, Reg. No. 43,418; Charles D. Niebylski, Reg. No. P-46,116; and Deborah A. Reitlin, P-45,904 as my attorneys to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith.

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Sixth Floor
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Washington, D.C. 20005 U.S.A.

We hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by 35 U.S.C. § 112, first paragraph, I/we acknowledge the duty to disclose material information as defined in 37 CFR § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

(U.S. Application Serial No.) (U.S. Filing Date) (Status--patented, pending, abandoned,

(U.S. Application Serial No.) (U.S. Filing Date) (Status--patented, pending, abandoned)

DECLARATION FOR PATENT APPLICATION

Attorney's fee letter - 24-500
Page 1 of 4

We hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and I further that these statements are made with the knowledge that willful false statements and the like are made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statement may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor: Meir SHINITZKY

Inventor's Signature M. Sherry Date 10/17/00

Residence: Derech Haanim Street 20, 46910 Kfar Shmarvahu, ISRAEL

Country of citizenship: ISRAEL

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Full name of second inventor: Michael DECKMANN

Investigator's Signature

July 4 2000

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STATEMENT OF CITIZENSHIP: GERMANY

2. The address of your residence

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Received: 8/29/00 3:13AM;
REINHOLD COHN & PARH.

972 3 7109407 -> Nath & Associates; Page 2

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REINHOLD COHN & PAR. 972 3 5663782

08/23 '00 16:13 NO.571 04/05



DECLARATION FOR PATENT APPLICATION

Attorney Docket: 24259
Page 1 of 2

As a below-named inventor(s), I/we hereby declare that:

My/Our residence(s), post office address(es) and citizenship(s) is/are as stated below next to my/our name(s).

I/we believe I/we am/are the original inventor, first and sole (if only one name is listed below) or the original, first and joint inventors (if plural names are listed below) of the subject matter which is claimed, and for which a patent is sought on the invention entitled:

SKIN TEST FOR SCHIZOPHRENIA

the specification of which: (check one)

() is attached hereto.

[X] was filed on 7 December 1998, as Serial No. PCT/IL98/00592,

and was amended on _____ (if applicable).

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

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Prior Foreign Applications:

<u>Application No.</u>	<u>IL</u> (Country)	<u>7 / Dec. / 1997</u> (Day/Month/Year Filed)	<u>(X) []</u> Yes No
<u>Application No.</u>	<u> </u> (Country)	<u> </u> (Day/Month/Year Filed)	<u> </u> Yes No
<u>Application No.</u>	<u> </u> (Country)	<u> </u> (Day/Month/Year Filed)	<u> </u> Yes No

We hereby appoint Gary M. Nath, Reg. No. 26,965; Harold L. Novick, Reg. No. 26,011; Todd L. Juras, Reg. No. 40,669; Lee C. Reiman, Reg. No. 41,827; Gerald L. Meyer, Reg. No. 41,196; Joshua S. Goldberg, Reg. No. 44,126; David Milligan, Reg. No. 42,893; David P. Murphy, Reg. No. 22,731; Paul A. Fischer, Reg. No. 43,418; Charles D. Wieblyski, Reg. No. P-46,116; and Deborah H. Yellin, P-45,904 as my attorneys to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith.

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We hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by 35 U.S.C. § 112, first paragraph, I/we acknowledge the duty to disclose material information as defined in 37 CFR § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

<u>(U.S. Application Serial No.)</u>	<u>(U.S. Filing Date)</u>	<u>(Status--patented, pending, abandoned)</u>
<u>(U.S. Application Serial No.)</u>	<u>(U.S. Filing Date)</u>	<u>(Status--patented, pending, abandoned)</u>

Dr. Michael Deckmann
22. 8. 2000

Michael Deckmann

=> REINHOLD COHN & PAR , TEL=972 3 5663782 08/28 '00 19:27

REINHOLD COHN & PARH.

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Docket No. 24259



REINHOLD COHN & PARH. 972 3 5663782 08/23 '00 16:13 NO.571 03/05

DECLARATION FOR PATENT APPLICATION

Attorney Docket: 24259
Page 2 of 3

I, hereby declare that all statements made herein or in my knowledge and true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are made and punishable by law, in applications or oaths, under 35 U.S.C. § 113; and that such willful false statements may invalidate the validity of the application or any patent issued thereon.

Name of Inventor: WILHELM SPITZERInventor's Signature: WSPAddress: Borchgrevink Street 23, 10639 Berlin GermanyCountry of Citizenship: GermanyPost Office Address: same as aboveTitle (name of second inventor): WILHELM SPITZERInventor's Signature: WSP Date: 23.8.2000Residence: 10639 Berlin GermanyCountry of Citizenship: GermanyPost Office Address: same as residence